Self-Thinning in *Ocimum basilicum* Grown at Three Soil Fertility Levels With and Without Mycorrhizal Inoculum

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To investigate whether mycorrhizal status affected the course of self-thinning of plant populations grown over a range of soil fertility, stands of *Ocimum basilicum* were established at three levels of soil fertility and two sowing densities on a soil-based potting mix that was either pre-heated to kill fungi, or not heated. Sampling of roots from the pre-heated soil mix failed to find mycorrhizal infection at any subsequent harvest, while roots from the non-heated soil showed mycorrhizal infection by second harvest. Self-thinning lines for shoot biomass at each fertility level did not differ in slope; however the line for the highest-fertility level was significantly lower in elevation (intercept) than a pooled line for the two lower-fertility levels. Self-thinning lines for shoot biomass showed no effect of mycorrhizal status: the relative position of thinning lines due to fertility level was the same for populations in both the mycorrhizal and non-mycorrhizal treatments. Self-thinning lines for root biomass differed significantly in slope. The root thinning line for the highest-fertility level generally lay under the line for the lowest-fertility level, while the line for the intermediate-fertility level crossed both lines.

This is the first reported case of populations from the highest-fertility level thinning along the lowest line on a biomass — density plot; in previous experiments, either populations from all fertility levels thinned along a line of common slope and intercept, or populations from the lowest-fertility level thinned along the lowest line. While root and shoot competition were not measured directly in this experiment, examination of root and shoot growth suggested that root competition was not the major determinant of the position of self-thinning lines. However shoot competition increased most quickly as shoot biomass accumulated in the stands grown at the highest-fertility level. The canopy volume required to support given shoot biomass was greatest in populations grown at the highest-fertility level, and this difference accounted for the separation of self-thinning lines on the shoot biomass — density plot.

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Introduction

The presence of neighbours in even-aged plant monocultures results firstly in a restriction of plant growth at high densities, and may result ultimately in death of suppressed plants (Shinozaki and Kira 1956, Yoda *et al.* 1963). These effects of competition are attributed to resource depletion caused by neighbours. When stands show this density-dependent mortality, or self-thinning (Yoda *et al.* 1963), increases in mean biomass (B, g/m^2) are related to decreases in mean density (N per m^2) by the relationship

$$\log B = K - \beta \log N \qquad (1)$$

where K is the intercept and β the slope (Yoda *et al.* 1963; Weller 1987). Initially, it was argued that β had an ideal value ($= -0.5$) (Yoda *et al.* 1963, White 1985); more recent work has shown that β takes a wider range of values (Weller 1987; Lonsdale 1990).

Since competition for resources is presumed to be the cause of self-thinning, vary-

ing the levels of resource supply might be expected to affect the process. Where light levels have been varied, shaded populations have thinned along lower lines (lower K and/or β, eqn. 1) than controls in full light in all cases (Kays and Harper 1974; Hutchings and Budd 1981; Westoby and Howell 1981, 1982; Lonsdale and Watkinson 1982, 1983; Dunn and Sharitz 1990).

For nutrients, however, two major results have emerged. Yoda *et al.* (1963) reported that stands of *Erigeron canadensis* grown with different levels of fertiliser supply thinned along a single line, of common intercept and slope (but see reanalysis of this result by Weller 1987). The rate at which populations traversed the common self-thinning line was directly proportional to fertility level: populations grown with the highest level of fertilizer supply traversed the self-thinning line the fastest. This effect of nutrient supply on self-thinning — regulating the speed of progression along a common self-thinning line — was also seen in two subsequent experiments. Mixed and pure populations of *Raphanus sativus* and *Brassica napus* grown at a range of fertility levels thinned along a biomass — density line of common slope and intercept (White and Harper 1970), as did mixed populations of *Sinapsis alba* and *Lepidium sativum* (Bazzaz and Harper 1976).

The other major result achieved has been a lower thinning line at lower levels of nutrient supply. Populations grown at lower levels of nutrient supply thinned along lines of reduced slope and/or intercept than controls grown at higher levels of nutrient supply for *Fagopyrum esculentum* (Furnas 1981), *Trifolium subterraneum* (Morris and Myerscough 1985) and *Ocimum basilicum* (Morris and Myerscough 1991).

These contrasting results may reflect important differences between stands in the mechanism of competition. However, detection of any such differences from the studies performed to date is confounded by the differences in methodology between studies (Table 1). These differences involved the growing medium used, the species used, the way in which nutrients were supplied, and (in the work of the present author), the presence or absence of mycorrhizae.

TABLE 1

Differences in methodology between studies where populations grown at a range of soil fertilities thinned along a line of common slope and intercept (common line), and studies where populations grown at a lower level of soil fertility thinned along a line of lower slope and/or intercept (lowered line) than controls grown at a higher fertility.

	common line	lowered line
growing medium	soil or soil-based	sand, perlite
species used	*E. canadensis* *R. sativus* *B. napus* *S. alba* *L. sativum*	*F. esculentum* *T. subterraneum* *O. basilicum*
nutrients supplied	solid form, mixed in medium	solution, regularly applied
mycorrhizae[1]	present	absent

1. in experiments of the author.

This paper investigates the last of these differences in methodology ie presence or absence of mycorrhizae. Plants of *O. basilicum* in the work of Morris and Myerscough (1991) were not mycorrhizal (and populations grown with reduced nutrient supply thinned along lower lines than full-nutrient controls). However in preliminary experiments with the same species on soil, infection by vesicular-arbuscular (VA) mycorrhizae did occur.

There are a number of possible mechanisms by which mycorrhizae might influence the course of competition (Allen and Allen 1990). The effect of mycorrhizae on uptake of nutrients is well-known: in addition to affecting uptake, mycorrhizae affect the location of nutrient depletion zones by shifting uptake away from the root (Jakobsen *et al.* 1992). Another possible mechanism is in transfer of materials (nutrients, carbohydrates) via hyphae between plants (Heap and Newman 1980, Chiariello *et al.* 1982, Read *et al.* 1985, McGee 1990). If this mechanism operated so that suppressed plants received material subsidies from dominant plants, this could well have affected the pattern of self-thinning by slowing down mortality in mycorrhizal stands.

So to test whether VA mycorrhizae had any effect on the pattern of self-thinning followed by populations grown at a range of soil fertilities, an experiment using *O. basilicum* grown at several fertility levels and in the presence or absence of mycorrhizae was established.

Methods

Growing methods and experimental design

A 7:3:2 mix of loam:peat:sand (formula for John Innes mix used by White and Harper 1970 and Bazzaz and Harper 1974) was used as potting media. Populations were established by sowing seeds of *O. basilicum* directly onto a thin layer of sand over the substrate in pots of 29 cm diameter and with a soil depth of 10 cm. Sowing densities were 1,321 or 3,300 seeds/pot (equivalent to 20,000 or 50,000 plants.m^{-2} — hereafter referred to as lower and higher densities). A perspex template was used to establish a hexagonal arrangement of seeds at the lower density; seeds were sprinkled as evenly as possible onto the substrate at the higher density, where use of a template was not feasible. Pots were watered as required, and kept in a glasshouse, where maximum light levels ranged from 1,600 mmol.cm^{-2}.s^{-1} early in the experiment to 1,000 mmol.cm^{-2}.s^{-1} by the end. As the plants grew *c.* 10 cm above the sand, a collar of 70% shade cloth was added around each pot and up to *c.* 2.5 cm below canopy height to reduce edge effects. As growth continued, successively higher collars were added. Three levels of soil fertility were established by adding 3, 6 or 12 g of macronutrients and 2.5, 5 or 10 g of micronutrients per pot (referred to hereafter as F1-, F2- and F3-fertility levels respectively). Macronutrients were supplied as Osmocote^{R} 270-day controlled release fertiliser (Sierra Chemical Co., Castle Hill, Australia), and micronutrients as Garden King Trace Elements^{R} (Retec Ltd., Murarrie, Australia). Nutrients were spread in a single layer *c.* 0.5 cm under the seed, to avoid inhibition of mycorrhizal infection by high nutrient levels in the bulk soil. The peat used lowered the pH of the potting mix, and so lime (70 g per pot) was added so that the pH of a filtrate of the saturated soil was >6. For the non-mycorrhizal treatment, elimination of mycorrhizal inoculum from the soil mix was achieved by steam-heating of soil to 80°C for 30 minutes (Sylvia and Schenck 1984). Heated soil was let stand for two weeks after treatment to allow soil microflora to re-establish. For the mycorrhizal treatment, the soil mix was not heated, to allow natural infection of roots from inoculum present in the soil. Pots used in the experiment were sterilised in dilute bleach, rinsed in distilled water, and elevated off the bench on inverted pots treated similarly. Six additional replicate pots were sown at the lower density, with no added nutrients (F0-fertility level), three on pre-heated and three on unheated soil.

The full experimental design was two densities x three fertility levels x two mycorrhizal treatments x four harvests x three replicates sown in randomised blocks containing factorial combinations of all treatments. Sowing occurred on 25 – 26 February 1991. The replicates for the fourth harvest became badly infected by a spray-resistant strain of *Botrytis*, so data are presented for only the first three harvests (weeks 6, 9 and 12) for the

F3–, F2- and F1-fertility levels. The pots from the F0-fertility level were unaffected by the *Botrytis* infection, and were harvested in week 15.

Plants were sprayed as required with Rovral (May and Baker, West Footscray) and Fongarid (Bayer Australia, Sydney) against *Fusarium* and *Botrytis*; Kelthane (Hortico (Australia), North Laverton) against red spider; and Foliomat (Bayer) against insect larvae. (While Rovral (active ingredient Iprodione) can inhibit mycorrhizal infection in some cases, West *et al.* (1993) found no effect of this spray in a glasshouse experiment with *Vulpia ciliata* spp. *ambigua*. Mycorrhizal infection of basil roots still occurred in the experiment reported here, despite its use on five occasions over the 12 weeks).

Sampling

At harvest a circular quadrat was positioned in the centre of each pot (using PVC pipe, internal diameter either 6.2 cm or 10.3 cm; the smaller quadrat was used for early harvests when densities were high, and the larger quadrat at later harvests as densities fell). Plants with stems rooted in the quadrat were cut off at soil surface. A random sample of ten individuals was selected from the quadrat population and scored for height, leaf number and total area of the laminae using a Lambda Instruments Corporation model LI 3000 (Lincoln, Nebraska). Mean leaf area per plant and total leaf area per quadrat were calculated, and used to estimate Leaf Area Index (LAI) (total leaf area per quadrat/ quadrat area) and Leaf Area Ratio (LAR) (mean leaf area per plant/ mean plant dry weight). As a measure of size variability within populations, the Coefficient of Variation (CV) of plant height for each sample was calculated (standard deviation/mean).

The pipe was pushed into the soil and used to extract a soil core. Root material was separated from the substrate by hand, after washing in a 2–mm sieve and subsequent flotation. Root length was determined on a root subsample using an Image Analysis system (Skye Instruments, United Kingdom); the relationship between length as given by Image Analysis and known length of cotton and root samples was found to be quadratic ($r^2 = 0.9986$), and so actual length of root samples was calculated by solving the equation. Shoot and root material (main root sample and root length sub-sample) were dried in a convective oven at 80°C for 24 h and weighed. Root length per plant was calculated from the total root length per sample (length:weight ratio of the subsample x the weight of the whole root sample) and density.

Sampling for mycorrhizal association

Soil samples for root extraction were taken from the border region adjacent to the root core sample, for all pots with pre-heated soil and selected pots with unheated soil. Roots were washed free from the soil, stained using the chlorazol black E method (Brundrett *et al.* 1984) and examined under the light microscope for the presence of vesicular-arbuscular mycorrhizae.

Nutrient levels in growing medium

The background level of nutrients in the unamended potting mix was examined using the procedure recommended for potting media by Warncke (1980). A 500 mL sample of the mix was saturated with distilled water, left to stand for 1.5 hours and filtered through a Buchner funnel under vacuum. Concentrations of selected nutrients in the filtrate were determined by inductively coupled plasma-optical emission spectrometry (Zarcinas and Cartwright 1983). Results were ($mg.L^{-1}$; mean ± S.E.): Ca = 231 ± 19; Mg = 120 ± 8 and P = 2.4 ± 0.3. These concentrations of Ca and Mg are rated optimal for growth by Warncke (1980); the concentration of P is rated low.

Data Analysis

Analysis of experiment

Comparison of treatment and interaction effects on density, shoot and root biomass, LAI, LAR and CV of plant height was made by Analysis of Variance (ANOVA).

Before analysis the homogeneity of variances in the raw data was checked by Cochran's test, and transformation used if necessary to achieve homogeneity. Missing values were replaced by cell means, and the degrees of freedom reduced accordingly. The full model (used to analyse biomass, LAI and LAR) included harvest, fertility level, density and mycorrhizal treatment as fixed factors. Comparison of treatment effects was by planned (orthogonal) comparisons of main effects or main effects in interactions, if interactions were significant (Keppel 1982). Trend analysis was used to analyse fertility effects, as nutrients were added at levels that represented equal increases along a (logarithmic) scale (Day and Quinn 1989, Keppel 1982). Density was analysed for fertility level, density and mycorrhizal effects at first harvest, and for fertility and mycorrhizal effects on the F1- and F2-fertility level populations from the lower density at second harvest.

Interpretation of data on size variability from within thinning populations is difficult, because the loss of plants by mortality affects the measure, and is concentrated in the smallest size-classes. Comparison of data on size variability was limited to the lower-sown density while these were still pre-thinning. Heteroscedasicity of variances precluded comparison of all three fertility levels at first harvest: comparison of the F1- and F2-fertility levels was made over the first and second harvests.

Since there were multiple ANOVAs conducted for the experiment, a sequential Bonferroni correction to significance levels was used to protect against increased risk of Type I error (Rice 1989).

Thinning lines — selection of data points

Once self-thinning begins, a subset of data points from each experiment must be selected *a posteriori* to fit eqn. (1) (Mohler, Marks and Sprugel 1978; Westoby 1984; Weller 1987). Inclusion of pre-thinning data points will affect the position of the line, and arguments about whether populations have begun to thin or not have been common (Weller 1987, Lonsdale 1990). Pre-thinning populations accumulate biomass with no or little change in density (ie progress vertically up the biomass — density plot). All populations from the higher-sown density showed a substantial decline from sown density (> 15%) at first harvest, and subsequently accumulated biomass while being subject to severe mortality. All populations from the higher-sown density were considered for inclusion in the calculation of thinning lines. Populations from the lower-sown density were also less than sown density at first harvest: however accumulation of biomass without substantial mortality was evident in stands from the lower-sown density grown at the F2- and F1-fertility levels, up to second harvest, and in one case third harvest. The variability in density of the pre-thinning populations represents the net effects of sowing, germination and establishment on density. The variability in density due to the above-mentioned factors was estimated by calculating the 95% confidence limits to a grand mean density for stands in each mycorrhizal treatment from the lower-sown density at first harvest (all fertility levels) plus second harvest (F2- and F1-fertility levels only) using log mean density as the variable. The 95% confidence limits were 9% of the grand mean for mycorrhizal populations and 6% for non-mycorrhizal populations. A decline in mean density of >10% from established density was required before populations from the lower-sown density were considered self-thinning.

Once thinning has commenced, data points may still be excluded from line-fitting, if other factors affect either mortality or biomass sufficiently to move the point away from the self-thinning line. A density-independent component of mortality operated in non-mycorrhizal populations at first harvest (see Results). If this occurs without a compensatory increase in biomass, data points so affected will be laterally displaced from the thinning line to lower densities. There is evidence of this at the F2- and F1-fertility levels, and so the non-mycorrhizal populations from the higher-sown density at first harvest were excluded from line-fitting (Fig. 2 b,c,e,f). Some data points from third harvest at the F3-fertility level were excluded from line fitting because they showed strong declines in both

biomass and density from the previous harvest (Fig. 2(a), (d)); these data points were outliers from the thinning lines ($P < 0.005$, see below).

Fitting of thinning lines

Thinning lines were fitted to selected data points on the log mean biomass (B) — log mean density (N) plot, for shoot and root biomass separately. (Results for total biomass closely followed those for shoot biomass, and are not presented). Since both variables were subject to variability, the functional relationship between them was described by the Major Axis of the data (fitted by Principal Components Analysis (PCA), Sokal and Rohlf 1981) following the convention adopted by earlier workers (Mohler, Marks and Sprugel 1978, Westoby 1984; Weller 1987). The r statistic for each line was used to report the strength of the relationship (Weller 1987). Limits to the slopes (L_1,L_2) were calculated (Sokal and Rohlf 1981).

It was difficult to detect whether the presence or absence of mycorrhizae affected self-thinning at each of the three fertility levels separately, because of the loss of one harvest and the subsequent low number of data points. To make comparisons possible, data points were pooled across fertility treatments, where the thinning lines and data for these treatments were not significantly different in slope or intercept. For shoot biomass, data from the F1- and F2-fertility levels were pooled, for comparison with the F3-level. Thinning lines for populations in the mycorrhizal and non-mycorrhizal treatments within each fertility level were calculated: (r was not significant for two of these data sets (Fig. 3(c)). However the data sets used for comparison of mycorrhizal effects were subsets of larger data sets with non-zero slopes, and so the calculated slopes (range -0.40 to -0.57) were taken as empirical descriptors of slope for the convenience of comparing treatment effects.

No comparison of mycorrhizal effects was attempted for root biomass, because of the more complicated pattern of thinning (Fig. 2) and the low number of data points in some data sets.

Root —shoot allometry

Allometric relationships of the form $\log y = b + m \log x$, where m = slope and b = intercept, were used to investigate patterns of root — shoot allocation. Biomass allocation was examined via shoot mass - root mass allometry, and relative size of resource-acquiring organs via leaf area — root length allometry. In both cases, as the variables were subject to both variability and correlated errors (density was used to calculate each) Geometric Mean Regression (GMR) was used to describe the functional relationship between the two variables (Rayner 1987); limits (L_1,L_2) to the slope were calculated using the formula of Jolicoeur and Mosimann (1968) reported by Ricker (1984). Lines were fitted initially to data from the individual pots from all harvests in each density x fertility level x mycorrhizal status combination, to give a (maximum) possible 12 lines.

Biomass — Canopy Volume relationships

Since differences in the biomass contained in given canopy volume can account for some of the differences observed between thinning lines, log mean shoot biomass (B) was plotted against log mean canopy volume (V) (which was estimated as mean plant height (Lonsdale and Watkinson 1983)). Allometric relationships were used to investigate biomass packing in the same populations as were selected to fit thinning lines for shoot biomass. The Major Axis of the data was fitted to describe the functional relationship between B and V (errors were uncorrelated in the variables).

Thinning lines were calculated for log V— Log N data for comparison with thinning lines calculated in terms of log B— log N, using the same set of data points and the same methods of line fitting.

Comparison of fitted lines

For lines fitted by either PCA or GMR, heterogeneity of slopes was tested by the maximum likelihood method proposed in Harvey and Mace (1982) (Rayner 1985). To do this, the data were rotated on axes so that the new origin was the bivariate mean of the pooled data, and the new X-axis was the (weighted) pooled slope. The test compares the r's from the rotated data sets: if the slopes are parallel, r approaches zero in all sets.

To test for differences in elevation from the common pooled slope, an ANOVA of the residuals after rotation was used (Clutton-Brock and Albon 1980; Harvey *et al.* 1980). The residuals measure the distance of the data points from the common pooled slope along the minor axis. Rejection of the null hypothesis indicates significant differences in elevation of the data sets (along the minor axis) from the common slope, and that different functional relationships apply to the data sets being compared. This test is analogous to (but not exactly the same geometrically as) the test for differences in intercepts in Analysis of Covariance (ANCOVA). (Use of ANCOVA to compare elevations in data sets where both variables are subject to variation can lead to an increased risk of Type I error (Huitema 1980)).

Lines not differing significantly in slope or intercept were pooled; the probability that differences between lines involved in pooling could have arisen by chance is reported to indicate the strength of differences between such lines. Suspected outliers from both thinning and allometric lines were tested by Grubb's test (Sokal and Rohlf 1981), using residuals after rotation.

Results

Mycorrhizal infection

No VA mycorrhizae were detected in roots from plants grown on pre-heated soil, at any harvest (Table 2). Mycorrhizal infection was detected in roots of plants grown on unheated soil at 6 weeks, in some pots; widespread infection of roots in all pots with unheated soil was evident by 9 weeks (Table 2).

Table 2

Results of sampling for vesicular-arbuscular mycorrhizae at each harvest (n = number of pots sampled).

Harvest	Soil treatment	n	Number of pots with VA mycorrhizae
6 weeks	unheated	6	2
	heated	18	0
9 weeks	unheated	18	18
	heated	18	0
12 weeks	heated	18	0

Germination, establishment and pre-thinning

At the higher-sown density, plant numbers were less than sown density at first harvest (52 – 83% of sown density in the mycorrhizal treatment and 40 – 57% in the non-mycorrhizal treatment), and numbers declined between each subsequent harvest (Fig. 1(a), 3).

At the lower-sown density, populations were also less than sown density at first harvest (78 – 96% of sown density in the mycorrhizal treatment and 76 – 79% in the non-mycorrhizal treatment, Figs. 1(b), 3). Substantial mortality (> 10% of established, see

Methods) by second harvest was only observed in the F3-fertility level stands at the lower-sown density; populations at the F1- and F2- levels showed little change in density while increasing in biomass between first and second harvests (Figs. 1(b), 3(b,c)). Most of the stands from the lower-sown density treatment at the F1- and F2-fertility levels did show substantial mortality by third harvest. The populations sown at the lower-density at the F0-fertility level and harvested in week 15 showed self-thinning (Fig. 4(b,c)).

Established densities at first harvest were significantly lower for populations in the non-mycorrhizal treatment than those in the mycorrhizal treatment ($P < .01$). This reduction was density-independent, being apparent at both sowing densities (Figs. 1, 3), and continued to be evident in the pre-thinning populations sown at the lower density at second harvest ($P < 0.05$).

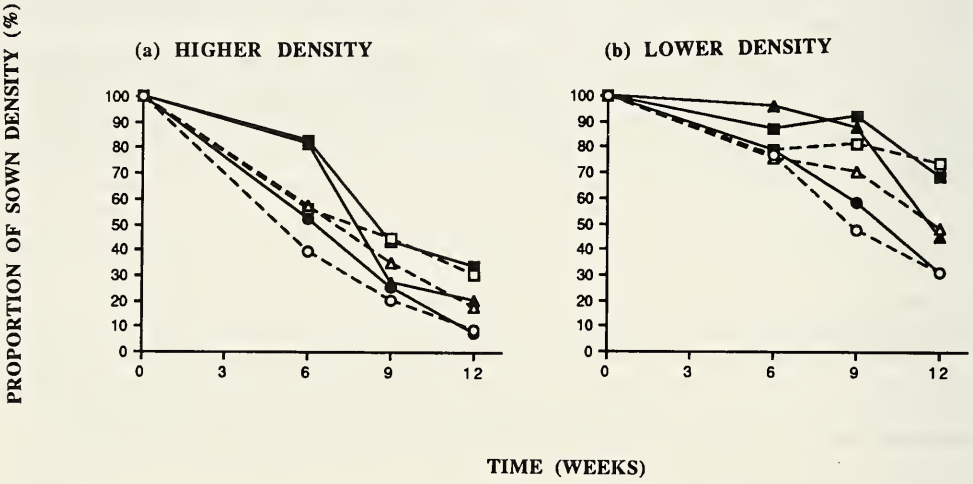

Fig. 1: Proportion of *O. basilicum* plants surviving at each harvest from the (a) higher density and (b) lower density populations in the mycorrhizal (closed symbols, solid lines) or non-mycorrhizal (open symbols, broken line) treatments grown at the F3- (●, ○), F2- (▲, △) or F1- (■, □) fertility level.

Biomass

Shoot biomass was significantly affected by harvest, mycorrhizal treatment (non-mycorrhizal > mycorrhizal) and soil fertility level (Fig. 2(a)). The relationship between shoot biomass and (ln) fertility level was best fitted by a quadratic relationship; the F2-fertility level yielded significantly more shoot biomass than the F1-, but the additional nutrients available in the F3-treatment did not increase biomass further (Fig. 2(a)).

Root biomass was significantly affected by harvest, and soil fertility level in interaction with mycorrhizal status (Fig. 2(b)). There was a linear increase in root biomass with soil fertility level in the non-mycorrhizal treatment, but no effect of soil fertility level on root biomass in the mycorrhizal treatment.

In the populations grown at the F0-fertility level and harvested in week 15, mycorrhizal stands had about 1.5 times the shoot biomass of non-mycorrhizal stands; root biomasses were similar in the two treatments (Fig. 4(b,c)).

Self-thinning

For shoot biomass the populations from the three fertility levels thinned along lines of similar slope (Fig. 3(a-c)); testing for heterogeneity of slopes showed differences were

non-significant ($P > 0.5$). The lines for the F1- and F2-fertility treatments were close, and not significantly different in elevation from a common slope ($P > 0.9$), so a pooled line was calculated (Fig. 4(a)). The line for the F3-fertility treatment lay *c.* 0.10 – 0.12 log units below the pooled F1 + F2 line (Fig. 4(a)); the elevation of these two lines from a common slope was significantly different (one-way ANOVA of residuals, $P < 0.001$). Pre-thinning populations from the lower-density treatment at the F1- and F2-fertility levels passed beyond the thinning line for the F3-stands at harvests 2 and 3 (Fig. 3(a - c)).

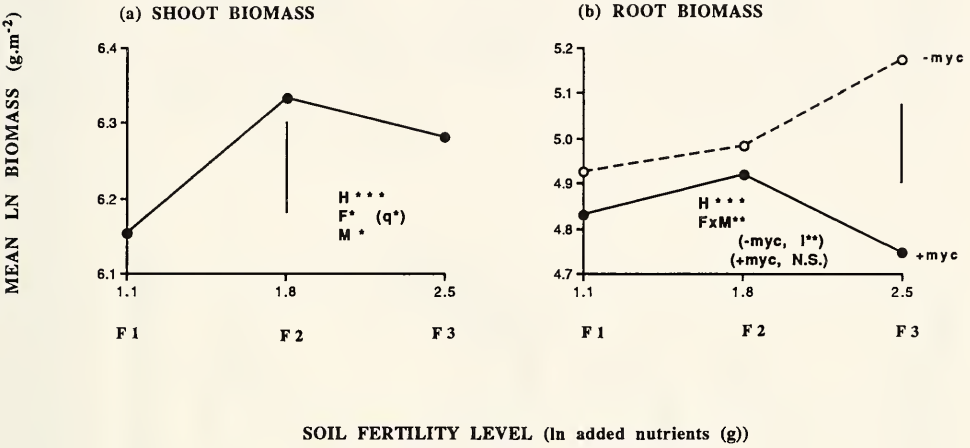

Fig. 2: Biomass (geometric means) plotted against fertility level for (a) shoot biomass and (b) root biomass. Treatment means for each fertility level shown in (a) were averaged over all harvests, densities and mycorrhizal treatments, and in (b) over all harvests and densities for populations in the mycorrhizal (+myc) and non-mycorrhizal (-myc) treatments. Significant terms only in the ANOVAs of (ln) shoot and (ln) root biomass are shown as main effects (harvest (H), fertility level (F), mycorrhizal treatment (M)) or interactions (FxM). The relationship between biomass and fertility level as given by trend analysis is shown (in brackets after F or FxM) as not significant (N.S.), linear (l) or quadratic (q). Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Vertical bar gives the Critical Value for comparison between two means (= $t_{0.05}$ x Standard Error of Comparison).

The slopes of the self-thinning lines fitted to root biomass — density data from the F1-, F2- and F3-fertility treatments were significantly different ($P < 0.01$) (Fig. 2(d-f)), with slopes becoming more negative in the order F2 > F1 > F3. The relative position of the three lines on the root biomass — density plot was complex, with the line for the F3-fertility treatment lying under the F1- line; the F2- line cut across both of the other two lines (Fig. 4(b)).

Mycorrhizal treatment had no effect on the elevation of thinning lines from a common slope, either as a main effect or in interaction with fertility level (two-way ANOVA of residuals from four lines in Fig. 4(c)). Non-significance of the interaction ($P > 0.25$) means that the relative position of the thinning lines for shoot biomass was the same for both mycorrhizal and non-mycorrhizal treatments across the range of fertility levels analysed (Fig. 4(c)), with the F3-line lying under the pooled F1+F2-line irrespective of mycorrhizal status. Non-significance of the main effect ($P > 0.25$) means that mycorrhizal status did not significantly affect the position of thinning lines for shoot biomass within each fertility level.

The single shoot biomass — density data point for populations in the mycorrhizal

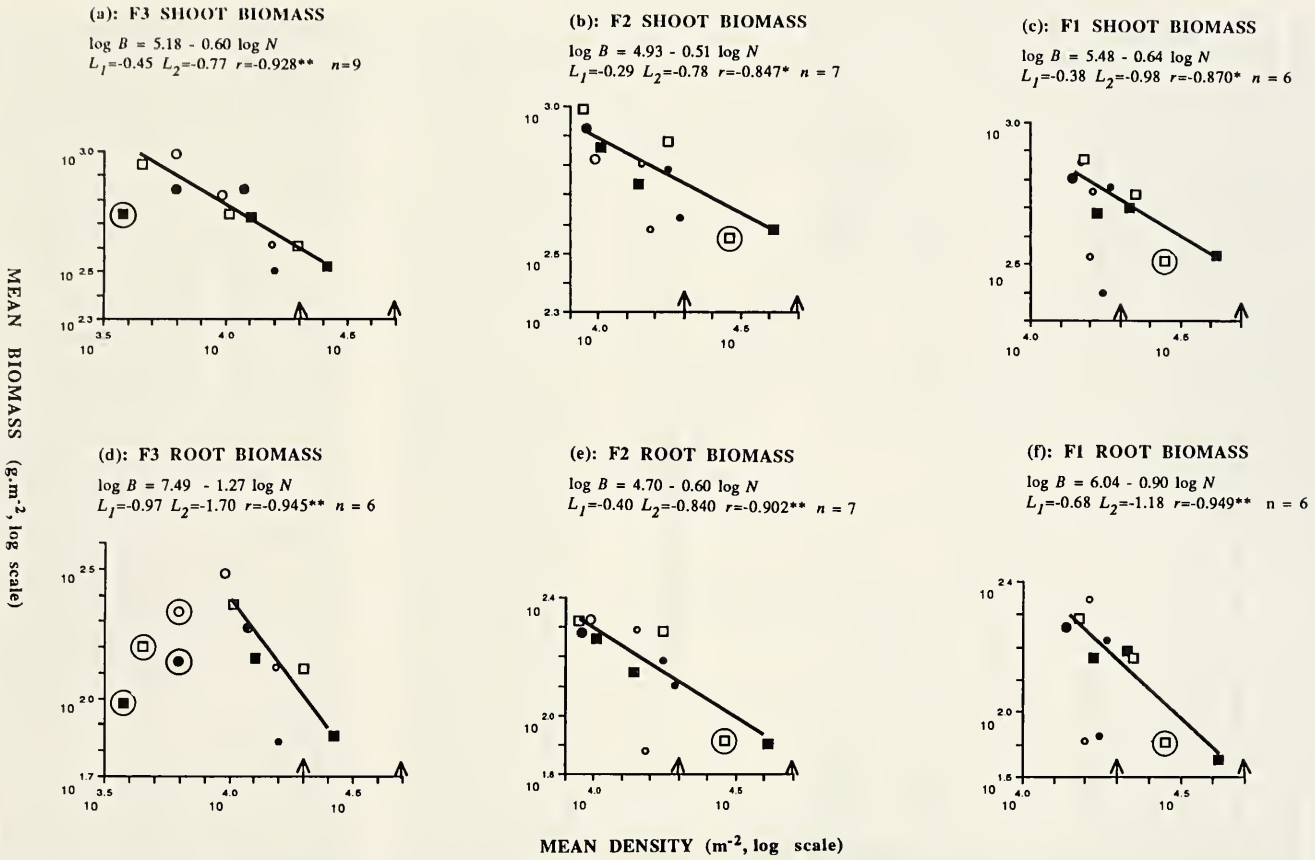

Fig. 3: Biomass (*B*) - density (*N*) relationships for shoot biomass (a - c) and root biomass (d - f) of populations of *O. basilicum* from the mycorrhizal (closed symbols) or non-mycorrhizal (open symbols) treatments grown at the F3- (a, d), F2- (b,e) or F1- (c,f) fertility level and sown at the higher (■,□) or lower density (●,○). Equations for self-thinning lines (__) are shown, with limits to slope (L_1,L_2), correlation coefficient (r) and sample size (n). Data points from pre-thinning populations are shown at smaller font size; circled data points were excluded from line-fitting (Methods); arrows on X-axis show sowing densitites. Significance levels: * P < 0.05; ** P < 0.01.

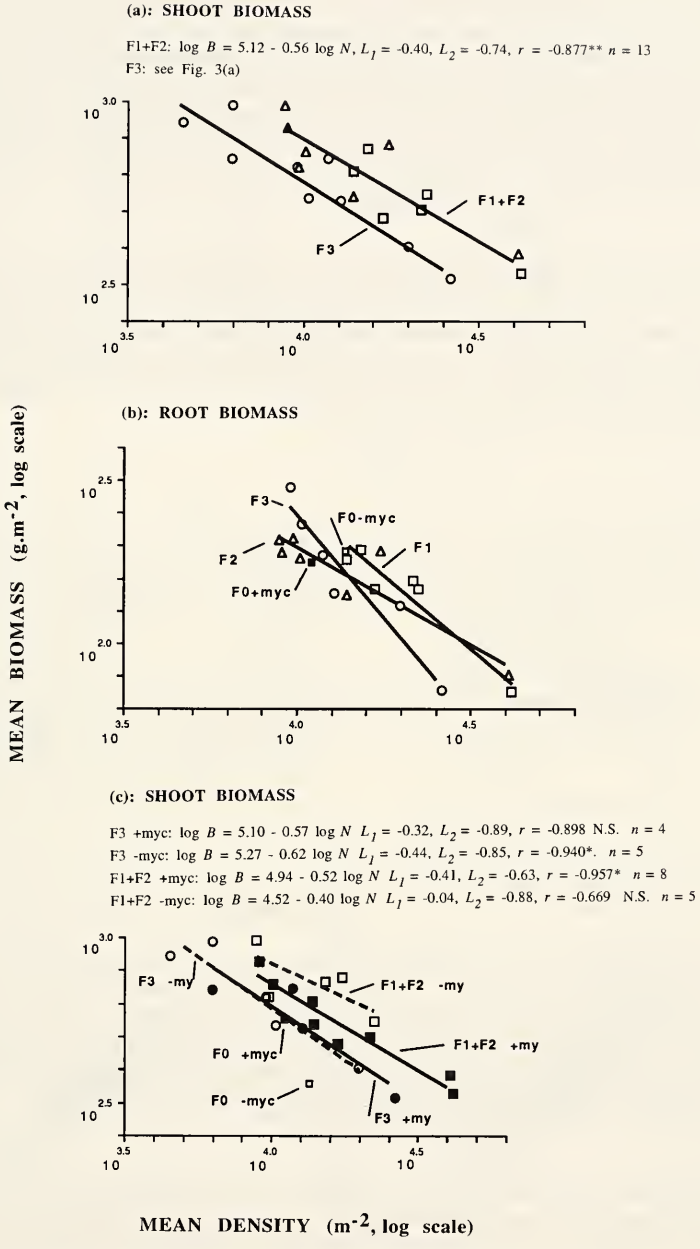

Fig. 4: Comparison of biomass (*B*) — density (*N*) relationships across fertility levels for (a) shoot biomass and (b) root biomass of *O. basilicum* grown the F3- (○), F2- (△), or F1- (□) fertility levels, and (c) for shoot biomass across fertility and mycorrhizal treatments (closed symbols, solid lines = mycorrhizal (+myc), open symbols, broken lines = non-mycorrhizal (-myc); F3- (●,○), pooled F1+F2- (■,□)). Only data from thinning populations used to fit lines are shown at F3-, F2- and F1-fertility levels. Single data points for populations grown at the F0-fertility level are shown in (b) and (c) for the mycorrhizal (F0+myc, □) and non-mycorrhizal (F0-myc, □) treatments. Self-thinning lines are shown labelled with (a,b) fertility level or (c) fertility level and mycorrhizal treatment. Equations of self-thinning lines not given in Fig. 3 are shown, with limits to slope (L_1,L_2), correlation coefficient (r) and sample size (n). Significance levels: N.S. not significant; * $P < 0.05$; ** $P < 0.01$.

treatment and grown at the F0-fertility level lay within the general region of data points from the other fertility levels, close to the thinning line for the F3-treatment (Fig. 4(c)); the corresponding data point for populations in the non-mycorrhizal treatment grown at the F0-fertility level lay about 0.15 log units below the thinning line for F3-fertility level populations (Fig. 4(c)). The root biomass - density data points for populations grown at the F0-fertility level lay within the general region of data points from the other fertility levels, with root biomass in the non-mycorrhizal treatment being slightly higher than root biomass in the mycorrhizal treatment (Fig. 4(b)).

Leaf Area

Stands carried significantly more leaf area as soil fertility increased, the relationship between (ln) nutrient level and (ln) Leaf Area Index (LAI) being linear (Fig. 5(a)). Stands in the non-mycorrhizal treatment carried significantly more LAI than stands in the mycorrhizal treatment (Fig. 5(a)). Allocation of biomass to leaf area (as measured by the Leaf Area Ratio (LAR)) significantly increased with soil fertility, the relationship between (ln) nutrient level and LAR being linear (Fig. 5(b)).

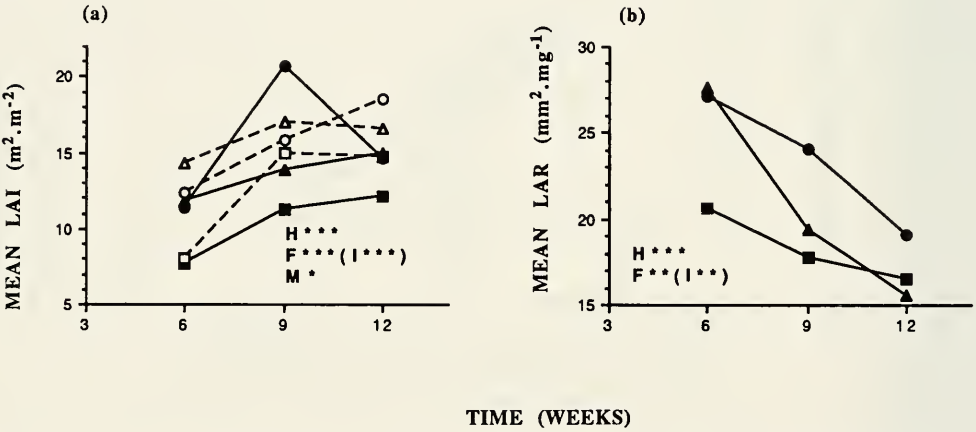

Fig. 5: (a) Mean Leaf Area Index (LAI) and (b) Mean Leaf Area Ratio (LAR) plotted against time for populations of *O. basilicum*. Symbols used are: (a) populations from mycorrhizal (closed symbols, solid lines) and non-mycorrhizal (open symbols, broken lines) treatments grown at the F3- (●, ○), F2- (▲, △) or F1- (■, □) fertility level; (b) populations grown at the F3- (●), F2- (▲) or F1- (■) fertility level. Densities were pooled within means shown in (a), and densities and mycorrhizal treatments were pooled within means shown in (b). Significant terms only in the ANOVAs of (ln) LAI and LAR (raw data) are shown as harvest (H), fertility level (F), or mycorrhizal status (M). The relationship between (ln) LAI or LAR and fertility level as given by trend analysis is shown (in brackets after F) as linear (l). Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Variability in size

Comparison of the Coefficient of Variation (CV) of plant height in pre-thinning populations showed that size variability within populations increased significantly with fertility level (Fig. 6(a)). Once thinning commenced, size inequality within populations generally decreased.

Root - shoot allometry

There was no detectable effect of soil fertility level on the allocation of total biomass to shoot or root biomass (Fig. 7(a)). There was no evidence of differences in slope between shoot mass - root mass relationships ($P > 0.5$), nor of differences in elevation from a common slope ($P = 0.25$). The overall treatment means of each of the three fertility

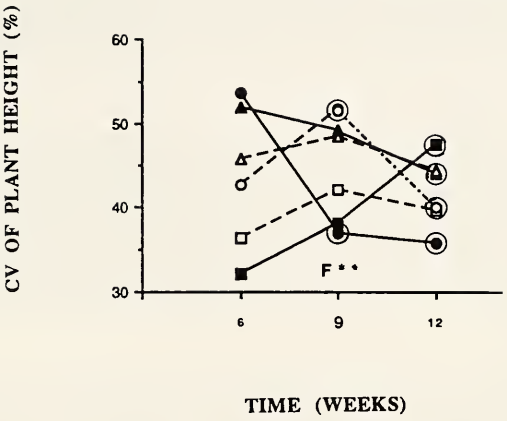

Fig. 6: Coefficient of Variation (CV) of plant height from populations of *O. basilicum* in the mycorrhizal (closed symbols, solid lines) or non-mycorrhizal (open symbols, broken lines) treatment sown at the lower density and grown at the F3- (○ , ●), F2- (▲ , △) or F1- (■ , □) fertility level. Data from thinning populations are circled. Significant terms only in the ANOVA of CV of plant height (for the F2- and F1-fertility levels at weeks 6 and 9) are shown as fertility level (F). Significance levels: ** $P < 0.01$.

levels for proportion of total biomass as root were 20 – 22%. A single line was fitted to data from all treatments (Fig. 7(a)). In the populations grown at the F0-fertility level, the proportion of total biomass as root was 24% in the mycorrhizal treatment and 35% in the non-mycorrhizal treatment (data not shown).

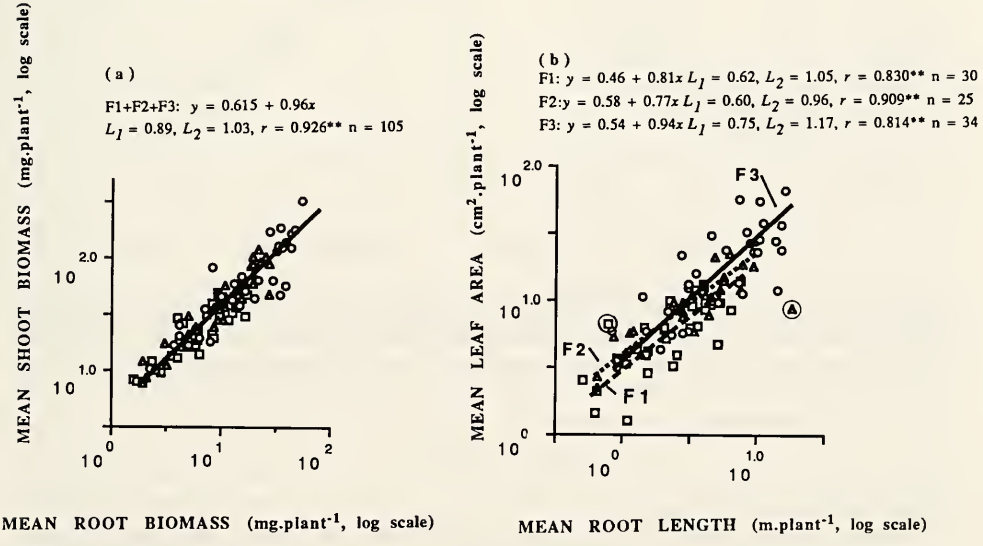

Fig. 7: Allometric relationships between (a) mean shoot biomass (y) and mean root biomass (x) per plant and (b) mean leaf area (y) and mean root length (x) per plant for populations of *O. basilicum* grown at the F3- (○), F2- (△) or F1- (□) fertility level. Lines and equations for allometric relationships are shown for (a) pooled F1+F2+F3 line, and (b) for each fertility treatment (F3- ——; F2- - - -; F1- – - –). Outliers in (b) are circled. Limits to slope (L_1,L_2), correlation coefficient (r) and sample size (n) are given. Significance levels: ** $P < 0.01$.

The leaf area carried per unit root length varied between the fertility treatments, with more leaf area per unit root length as soil fertility level rose. Slopes of the leaf area - root length relationships were homogeneous ($P > 0.1$); differences in elevation from a common slope of 0.85 were significant ($P < 0.005$). Trend analysis showed that there was a linear increase in the adjusted mean of the residuals for each fertility level, as fertility level increased.

Shoot Biomass - canopy volume

Populations grown at the F1- and F2-fertility levels had more shoot biomass in given canopy volume than those grown at the F3-fertility level (Fig. 8(a)). The shoot biomass - canopy volume relationships for the three fertility levels were linear on a log - log plot, and were not significantly different in slope ($P > 0.9$). The lines for the F1- and F2-fertility levels were very close, and not significantly different in elevation from a common slope ($P = 0.87$); the pooled F1+F2 line lay significantly above the line for the F3-fertility level ($P < 0.001$) (Fig. 8(a)).

Canopy volume - density

Thinning lines calculated in terms of canopy volume were quite close together, and showed no significant differences in slope ($P > 0.1$) or in elevation from a common slope ($P = 0.19$) (Fig. 8(b)).

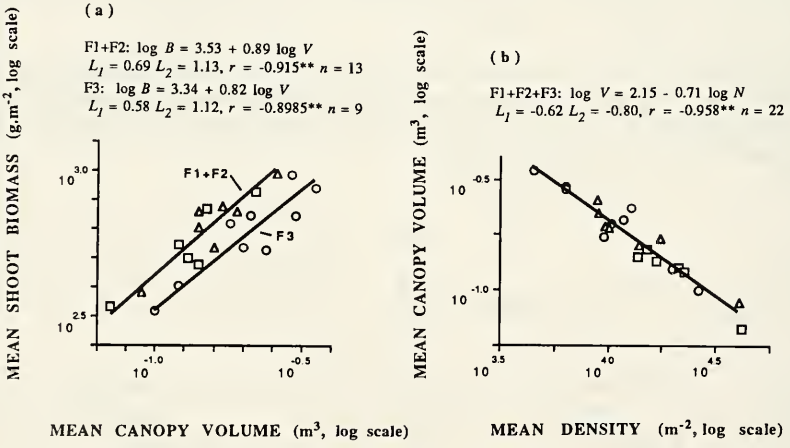

Fig. 8: Relationships between (a) mean shoot biomass (B) and mean canopy volume (V) and (b) mean canopy volume (V) and mean density (N) for populations of *O. basilicum* grown at the F3- (○), F2- (△) or F1- (□) fertility level. Lines and equations for (a) allometric relationships between B and V of the pooled F1+F2- and F3-fertility levels, and (b) pooled $V - N$ self-thinning line for populations from all fertility levels are shown. Limits to slope (L_1,L_2), correlation coefficient (r) and sample size (n) are given. Significance levels: ** $P < 0.01$.

Discussion

Mycorrhizal effects on self-thinning

The mycorrhizal status of populations grown over a range of soil fertilities had no detectable effect on the biomass — density relationships of those populations as they self-thinned, in this experiment. The relative position of thinning lines for shoot biomass remained the same across the range of fertility levels used (F1 – F3) , for populations in both the mycorrhizal and non-mycorrhizal treatments (Fig. 4(c)).

Some important qualifications should be added to this conclusion. Mycorrhizal infection of plants grown on unheated soil was slow to develop, only being evident in all pots from that treatment at second harvest (Table 2). Any possible effects of mycorrhizae on self-thinning may have been restricted to the latter part of the experiment, and may not have had time to become apparent. However, if a strong effect of mycorrhizae on self-thinning had been present in the experiment, it should have become apparent by the end, as between one-third to one-half of the progression of populations along self-thinning lines occurred between the second and third harvests (Fig. 3).

The range of soil fertilities achieved in the experiment must also be considered. The soil-based potting mix used in this experiment had good levels of some background nutrients; testing for levels of nutrients present in the mix gave values in the good — excellent range (Warncke 1980), before the addition of fertilizer. In addition, the growth of plants in the non-mycorrhizal treatment at the F1 – F3-fertility levels was better than that of plants in the mycorrhizal treatment, an effect that has sometimes appeared in other studies (Fitter 1977, West *et al.* 1993). Whether this was because pre-heating of the soil has had some unknown side effects on growth, such as mobilising nutrients, or there was a net cost to the plant of maintaining the symbiont (Peng *et al.* 1993) is unknown. Since the presence of mycorrhizae did not confer an advantage in growth in this experiment, repetition of the experiment, at a soil fertility level where mycorrhizal infection did confer such an advantage, may well give a different result.

The single data points from the F0-populations, if indicative of the self-thinning paths followed at this lower fertility level, support this view. Mycorrhizal infection conferred an advantage in (shoot) growth at the F0-fertility level, and populations from this treatment had greater biomass for roughly comparable thinning density than non-mycorrhizal populations (Fig. 4(c)). If this represents a treatment effect (rather than random variation), it would mean that at fertility levels lower than those used here, mycorrhizal populations would thin along a higher biomass — density line than non-mycorrhizal populations.

The conclusion that mycorrhizal status did not affect self-thinning at the range of soil fertilities used, followed from an analysis of data points selected by the investigator, and consequently some doubt could remain about its validity — other investigators might choose points differently. In particular, the position of the lines for the F2- and F1-fertility levels were determined by one data point at the high-density end, with no further data points for over half the length of the line (although pooling of the shoot biomass data for these two fertility levels overcame this problem somewhat). Exclusion of the first harvest data points for the non-mycorrhizal populations from the higher-density treatment, on the grounds of density-independent mortality, might not be justified (Fig. 3). It is not known what caused density-independent mortality at the establishment phase in this experiment. A density-independent reduction in established plant numbers below sowing density has been observed in a second experiment with *O. basilicum*, where a fungicidal soil drench was used at sowing (Morris, unpublished data). Germination and establishment of *O. basilicum* would appear to be sensitive to soil treatments such as pre-heating or drenching. Inclusion of the first harvest data points for the populations in the non-mycorrhizal higher-density treatment at the F1- and F2-fertility levels, while changing the parameters of the lines, did not alter the conclusion. For shoot biomass, slopes of the three lines were still homogeneous; the lines for the F1- and F2-fertility levels were still not significantly different in elevation, and when pooled gave a line of $\log B = 5.47 - 0.64 \log N$, which was still significantly higher in elevation than the line for the F3-treatment. Alternatively, using the individual biomass - density value for each pot (rather than harvest means) for analysis gives a greater spread of data points along each line, and more power in the comparison of fertility and mycorrhizal effects. The conclusion drawn from an analysis of individual pot data was still the same as that drawn from the analysis of harvest mean data reported here: mycorrhizal effects were not apparent, while fertility effects were.

Effects of soil fertility on self-thinning - the intensity of competition

Previous work has shown that mortality proceeds the fastest in populations grown with the greatest supply of nutrients (Yoda *et al.* 1963, White and Harper 1970, Bazzaz and Harper 1976, Furnas 1981, Morris and Myerscough 1985, 1991), and this occurred in the experiment reported here. Thus, on a time basis, and using the extent of mortality as the measure, the intensity of competition was greatest in the populations grown at the highest level of soil fertility.

On a biomass basis however, the intensity of competition can be judged by the biomass at which thinning commences, the biomass that thinning populations can subsequently support for a given density, and the ground area ($= N^{-1}$) surviving plants require to support given biomass. In the experiment reported here, populations grown at the highest-fertility level supported the least shoot biomass for a given thinning density, and the surviving plants from the highest-fertility level required the greatest ground area to support given shoot biomass (Fig. 4(a)). Thus, competition was most intense (on the basis of shoot biomass), in the populations grown at the highest-fertility level (as argued by Grime 1979).

This contrasts with the conclusion drawn by previous workers from experiments of the same design, using a soil-based growing substrate. White and Harper (1970) and Bazzaz and Harper (1976) concluded that the intensity of competition (on a shoot-biomass basis) was the same in all stands over the range of soil fertility used, since all populations thinned along a line of common slope and intercept. The result obtained in the experiment reported here also contrasts with that observed when a sand or perlite potting medium has been used, where competition has been the most intense in populations grown at the lowest levels of nutrient supply (on a shoot-biomass basis, Furnas 1981; on a shoot- and root-biomass basis, Morris and Myerscough 1985, 1991).

Thus the effect of soil fertility on self-thinning seen in the experiment reported here has not been observed before. It is of both theoretical and practical interest to know which of a number of populations grown at different soil fertilities will commence thinning at the lowest biomass. A model can be suggested from the data gathered from this and earlier experiments to explain the differing observations recounted above.

The population that begins self-thinning at the lowest biomass presumably does so because total competition is the most intense in that stand. Total competition has above-ground and below-ground components. The rate at which either form of competition intensifies as biomass accumulates depends on the biomass allocated to and architecture of resource-acquiring organs, the physiological activity of those organs (Goldberg 1990), and relative size inequality within the populations. The intensification of shoot or root competition (or an interaction between them) to levels sufficient to induce mortality would thus determine whether stands grown on the most-fertile or the least-fertile substrate began thinning at the lowest biomass, or whether stands from all fertility levels thinned at a common biomass. While the intensity of shoot and root competition was not directly measured in this or earlier experiments, an estimation of their relative importance can be made *post-hoc* by examining the allocation to and the dimensions of the organs of resource capture in the populations.

In the experiment of Morris and Myerscough (1991) plants at the two lower levels of nutrient supply grew more root, and less leaf, which was deployed in smaller canopies, than those grown at the highest level of nutrient supply. Morris and Myerscough (1991) argued that below-ground factors were the major determinant of the beginning and course of self-thinning in both of the lower-nutrient supply treatments.

In the experiment reported here, plants grown at all levels of soil fertility allocated the same amount of biomass to shoot or root growth (Fig. 7(a)). While conversion of biomass into leaves or roots did differ with soil fertility level, these differences were not correlated with the position of the self-thinning lines. Plants grown at the F1-fertility level required more root length to support given leaf area than those at the F2- or F3- (Fig.

7(b)). The extra root length required by plants at the F1-fertility level to support given leaf area can lead to intensified root competition, if the extra root is located close enough to neighbour's roots for depletion zones to overlap, and the total supply of nutrients is insufficient for the growth of all plants. So while the preconditions may have existed for the intensification of root competition as soil fertility level declined in this experiment, any such intensification (if present) was not sufficient to initiate self-thinning at the lowest biomass in the stands grown on the least-fertile substrate.

The evidence available suggests that shoot competition became most intense in the populations grown at the F3-fertility level as shoot biomass accumulated. Shoot biomasses achieved at the F2- and F3-fertility levels were similar (Fig. 2(a)); however, the deployment of this biomass in space (canopy architecture) differed markedly between the two fertility levels. While populations from all three fertility levels occupied the same canopy volume as they self-thinned (Fig. 8(b)), populations at the F3-fertility level required greater canopy volume to support given shoot biomass (Fig. 8(a)). Thus a given shoot biomass was deployed in the greatest canopy volume in stands grown at the F3-fertility level; this would lead to interference with neighbours at a lower biomass in the F3-stands than in the F1- and F2- stands (Grime 1979). The separation of thinning lines between the F3- and pooled F1 + F2-fertility levels on the shoot biomass density plot (Fig. 4(a)) was accounted for by the separation between the lines for these same treatments on the shoot biomass - canopy volume plot (Fig. 8(a)). This effect has been reported for *Helianthus annuus* plants grown at different levels of shading (Lonsdale & Watkinson 1983). Shaded populations required greater canopy volume to achieve given shoot biomass, and also thinned along lines of lower intercept on a shoot biomass - density plot.

The leaf area carried by populations increased linearly with fertility level, and this would contribute to an intensification of shoot competition, with suppressed plants being the most heavily shaded in populations growing on the most-fertile substrate. However this pattern developed more at second and third harvests (Fig. 6(a)); LAIs in the F2- and F3-stands were similar at first harvest. LAR, which measures biomass allocation to leaf area, increased linearly with fertility level, and again, this would contribute to an intensification of shoot competition from the F2- to the F3-stands, which both had similar shoot biomass. But also again, actual LARs achieved in the F3-stands only became clearly differentiated from those at the F2-fertility level at second and third harvests (Fig. 5(b)). Size inequality within pre-thinning populations increased with fertility level also, which would mean that suppressed plants were relatively smaller than dominants as soil fertility increased. However, the greatest increase in size inequality in pre-thinning populations was from the F1- to the F2-stands: size inequaltiy in pre-thinning F3-stands was comparable to that in F2-stands (Fig. 7).

I would propose that as plants grew in this experiment, root competition was not important in determining the relative position of thinning lines for shoot biomass. However, shoot competition per increment of biomass intensified most quickly in populations grown at the F3-fertility level, and while the amount of leaf area carried in the F3-stands contributed to this, canopy volume occupied per unit shoot biomass was the factor most consistently associated with this intensificaton over the whole of the experiment.

A more general model can be suggested from the results of this experiment. If populations are grown over a range of soil fertilities, thinning will commence at the lowest biomass in the population in which total competition intensifies the most per increment of biomass. Whether this is the stands grown on the most-fertile substrate (this experiment), or the least-fertile substrate (Furnas 1981, Morris and Myerscough 1985, 1991) or all stands commence thinning at the same biomass (White and Harper 1970, Bazzaz and Harper 1976) would depend on the rate at which shoot and root competition intensify per increment of biomass. This model is based on *a posteriori* correlations, and would require testing by experiment before gaining acceptance.

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